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Haemophilus influenzae type b carriage among 3- to 24-month-old Turkish children

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SUMMARY

There are few studies from developing countries on the epidemiology of *Haemophilus influenzae* (Hib) infections among infants and children. We set out to determine the prevalence of oropharyngeal Hib colonization among Turkish children younger than two years of age and to identify antimicrobial resistance among the isolates. A cross-sectional study was conducted on 818 healthy children and oropharyngeal secretions were sampled. The carriage rate of Hib was found to be 7·2% and this increased significantly with age. Carriage of Hib among 3- to 6-month-old children (3·5%) was higher than expected and was significantly higher among children who were passive smokers ($P=0·04$). Logistic regression analysis showed that breastfeeding status was the sole significant factor for colonization (OR 2·2, 95% CI 1·26–3·82). Antimicrobial susceptibility tests on 56 isolates of *H. influenzae* showed that 51·8% and 21·4% were resistant to trimethoprim–sulphamethoxazole and ampicillin respectively. Other notable resistances were to cefalexin (10·7%) and chloramphenicol (3·6%); no isolates were resistant to ceftriaxone.

INTRODUCTION

Haemophilus influenzae type b (Hib) is one of the most important bacterial pathogens that cause infections with high mortality and morbidity in children <5 years, most invasive infections occur in children <2 years of age. Hib colonizes nasopharyngeal and oropharyngeal mucosa and is transmitted from person-to-person by respiratory secretions of healthy children and adults. Carriage may be attenuated by several factors and Hib conjugate vaccines are highly effective in preventing invasive disease with this organism [1]. The widespread use of these conjugate vaccines in industrialized countries has led to the near elimination of invasive Hib disease among infants and children [2, 3]. In several countries, the conjugate vaccine has been shown to protect unvaccinated children by decreasing carriage among vaccinated children [4–6]. Meningitis and pneumonia due to Hib are important public health problems in developing countries but the cost of the conjugate vaccine is a major obstacle to its mass application. Indeed, there are few population-based epidemiological studies of Hib infection in developing countries [7–10].

Asymptomatic carriers have been recognized as the major source of Hib infection. Factors contributing to the epidemiology of this carriage are social and demographic and its risk factors may differ among various countries. The aim of this cross-sectional study was to identify the Hib carriage rate and risk factors for colonization among healthy Turkish children and to determine the *in vitro* activity of several antimicrobial agents against the isolates.

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METHODS

The study was carried out between January 1999 and June 2002 with the approval of the Ethical Committee of the Istanbul Medical Faculty. Informed consent was sought from all families before taking oropharyngeal swabs. The study encompassed 3- to 24-month-old children attending the Well Child Clinic of the Istanbul Medical Faculty Hospital. We planned to evaluate children in three age groups, 400 children aged 3–6 months, 207 children aged 7–12 months and 211 children aged 13–24 months. The number of children in each age group was calculated from data of the carriage rate of Hib in developing countries [carriage rates in these age groups were 5, 15 and 15% respectively with the 95% confidence interval (CI)] [7–11].

Families brought their children to the clinic for health checks and immunizations on a voluntary basis. Children who came to the clinic during the study period were selected consecutively for the study. Premature infants, children with acute infections, those receiving antibiotics and those with cleft palate were not included in the study. Participants were generally from middle-to-high income families. A questionnaire was sent to each parent to collect data on factors known to affect oropharyngeal carriage of Hib. These included household size, number and age of siblings, day-care attendance and number of smokers at home. Breastfeeding and vaccination status of each child was obtained from the health records of each child.

Vaccines in the Expanded Programme for Immunization (EPI) are supplied free of charge to all children by the Turkish Ministry of Health and this programme is followed in the Well Child Unit, however, Hib vaccine is not included. Information on this vaccine is given to all families attending the clinic, but is limited to those who can afford to purchase the vaccine. The PRT-T conjugated vaccine (SmithKline Beecham, Rixensart, Belgium; Pasteur Mérieux Connaught Lyon, France) was used for immunization of children at 2, 3 and 4 months of age.

Oropharyngeal secretions were collected from children using sterile cotton-tipped applicator swabs with tongue depressors to visualize the oropharynx. Swabs were inserted into both tonsils and posterior pharynx, rotated twice and withdrawn before placing in Stuart's Transport Medium (Venturi Transystem, Brescia, Italy) and transferred to the microbiology laboratory within 2 h. Each swab was inoculated on chocolate agar supplemented with 300 μg/ml of bacitracin to isolate *H. influenzae*. The plates were incubated in air containing 5–7% CO_2 for 24–48 h and colonies resembling *Haemophilus* spp. colonies (colony morphology, colour and odour) were selected for further tests. Organisms were identified as *H. influenzae* by their requirement of X and V factors (Oxoid, Basingstoke, UK). Haemolytic strains on trypticase soy agar containing horse blood was used to rule out *H. haemolyticus* which also requires X and V factors for growth. A conventional slide agglutination test with type-b specific anti-serum (Difco, Detroit, MI, USA) was used to confirm the identity of *H. influenzae* type b.

Table 1. *Characteristics of the children in the survey of* H. influenzae *type b colonization*

Characteristics	No of children (%)
Day-care attendance	16 (1·9)
Two or more siblings	415 (50·7)
Siblings attending day-care centre	270 (33·0)
Household with >4 persons	482 (58·9)
At least one smoker in household	322 (39·3)
Currently breastfed	516 (63·0)
At least one dose of Hib vaccine	615 (75·2)

Antibiotic susceptibility testing was performed by the disk diffusion method in Haemophilus Test Medium (HTM) under standard conditions according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) [12]. *H. influenzae* ATCC 49247 was used as a quality control strain. The following antimicrobials (Oxoid) were tested: ampicillin, ampicillin–sulbactam, amoxicillin–clavulanic acid, trimethoprim–sulphamethoxazole, cefalexin, ceftriaxone, cefuroxime and chloramphenicol. β-Lactamase activity was detected with nitrocefin disks (Becton Dickinson, Plymouth, UK).

SPSS for Windows, 10.0 (SPSS Inc., Chicago, IL, USA), was used for all statistical analyses. The Pearson χ^2 test and Fisher's exact test were performed to compare groups and logistic regression analysis was applied to evaluate possible risk factors.

RESULTS

Of 818 children, 56% were male. The characteristics of children are summarized in Table 1. The proportion of children attending a day-care centre was very low (1·9%) and 63% were receiving breast milk at the time of study. Three-quarters of all subjects had received at least one dose of Hib vaccine prior to the study.

Table 2. H. influenzae *type b carriage rate in different age groups*

Age group (months)	Colonization rate (%)	χ^2
3–24 ($n=818$)	7·2	$P=0·001$
3–6 ($n=400$)	3·5	$P=0·02$*
7–12 ($n=207$)	7·7	$P=0·04$**
13–24 ($n=211$)	13·7	$P=0·01$***

* 3–6 *vs.* 7–12 months; ** 7–12 *vs.* 13–24 months; *** 13–24 *vs.* 3–6 months.

Oropharyngeal swabs from 59 (7·2%) of the 818 children yielded *H. influenzae* type b. The carriage varied by age from 3·5% positive in the 3–6 months age group to 13·7% in the 13–24 months group (Table 2). The carriage rate increased significantly with age for all children ($P=0·001$). The prevalence of *H. influenzae* type b among the 7–12 months age group was significantly higher than that among the 3–6 months group ($P=0·02$). Carriage rates did not differ between girls and boys in the three age groups.

The overall vaccination rate was 75·2% and the proportion of colonized children was not significantly different between unvaccinated (8·4%) and vaccinated (6·8%) subjects. The number of vaccine doses did not appear to effect the colonization. The carriage rates for *H. influenzae* type b and the effect of risk factors are shown in Table 3. The carriage was significantly lower among currently breastfed children than among non-breastfed (10·2% *vs.* 5·4%) ($P=0·01$), but this difference was not statistically significant when the age groups were taken into consideration. The presence of at least one tobacco smoker in the household significantly increased the overall carriage rate in children (9% *vs.* 6%) ($P=0·04$). In the logistic regression analysis, only breastfeeding status was a significant factor for oropharyngeal colonization.

Fifty-six isolates of *H. influenzae* type b were examined for resistance to antibiotics. Resistance was highest to trimethoprim–sulphamethoxazole (51·8%) and ampicillin (21·4%). One isolate each was resistant to ampicillin–sulbactam, amoxicillin–clavulanic acid and cefuroxime. Cefalexin and chloramphenicol resistances were 10·7% and 3·6% respectively and none was resistant to ceftriaxone. Seven isolates showed β-lactamase activity and all were resistant to ampicillin.

DISCUSSION

The main finding of this study was that carriage of *H. influenzae* type b among infants and young children was higher than that reported from industrialized countries. The peak incidence was among the 13–24 months age group and the questionnaire survey suggested that passive smoking increased carriage among the 3–6 months age group while breastfeeding appeared to reduce carriage.

The carriage rate of *H. influenzae* type b among children <5 years from industrialized countries has generally been 3–5% [13, 14]. The children in this study were <2 years old and the carriage rate of 8·4% in unvaccinated children obtained is similar to that reported from developing countries (4·6–12·5%) [7–11]. An earlier study from Turkey reported a carriage rate of 2·1% among 430 children aged 0–24 months [15]. This difference between the surveys may be due to differences in the age distribution of the children as here we did not involve children younger than 3 months. According to our findings, the rates of colonization increased with age, especially after 12 months and this is consistent with reports from some developed countries [16, 17]. The Well Child Clinic provides services predominantly for children of health-care workers. This factor may have contributed to the higher carriage rate as this professional group would be more aware of the risks of infection and, thus, be more motivated to bring their children to the clinic. However, we did not collect specific data on parental occupation and so we cannot comment on the effect of occupation on colonization status.

There are few studies on antibiotic resistance of *H. influenzae* type b strains from developing countries. A study from China [18] reported a carriage rate of 1·0% and relatively low ampicillin resistance (4·8%) but high resistance to trimethoprim–sulphamethoxazole (77·1%). This is in marked contrast to the rates found here for these antibiotics (21·4 and 51·78% respectively).

Breastfeeding is thought to prevent attachment of *H. influenzae* to nasopharyngeal epithelial cells and has been associated with reduced colonization and risk of invasive Hib disease [10, 19, 20]. This was confirmed here as children currently breastfed had a significantly lower rate of Hib carriage than that of non-breastfed children. Exposure to tobacco smoke has also been associated with an increased risk for Hib disease [21, 22] and this was supported by the high carriage rate among passive smokers.

Table 3. H. influenzae *type b carriage rate (%) in children according to risk factors*

Characteristics	Yes (%)	No (%)	Adj. OR	95% CI	*P*
Currently breastfed ($n=516$)	5·4	10·2	2·2	1·26–3·82	0·005
Household: >4 persons ($n=482$)	6·8	7·7	1·51	0·51–4·48	0·45
Sibling at home: ⩾2 ($n=415$)	7	7·4	0·84	0·25–2·83	0·78
Sibling attending day-care centre or school ($n=270$)	7·4	5·3	0·93	0·41–2·16	0·88
Vaccination					
1 shot ($n=109$)	7·3	8·4	1·21	0·64–2·31	0·55
2 shots ($n=109$)	2·75	8·4	1·1	0·42–2·38	0·98
3 shots ($n=397$)	7·8	8·4	0·36	0·11–1·22	0·11
Smoking (at least one person) ($n=322$)	9	6	0·60	0·34–1·03	0·06

OR, Odds ratio; CI, confidence interval.

Despite its success in reducing carriage of Hib in industrialized countries [4–6], conflicting results on the impact of vaccination have been obtained from the developing world. For example, in Brazil, children in day-care centres who had received adequate vaccination were four times less likely to be carriers [23]. The impact of vaccination was less dramatic in populations with existing high carriage rates and an early peak incidence [16]. We did not find any difference in Hib carriage rate between the vaccinated and unvaccinated children and even in different age groups, vaccine dosage did not affect oropharyngeal colonization. This may be due to the low Hib vaccination rate in the population where these children live. On the other hand, although the vaccination rate was high in our study population, the number of children who received three doses of Hib vaccine was not high. Continued circulation of Hib in a population may cause shorter duration of vaccine effect on colonization in vaccinated children [16].

The presence of a sibling and day-care attendance have been identified as risk factors for Hib disease in several studies with colonization being higher among children in day-care centres and orphanages [24, 25]. A relatively small proportion of our children attended day- care centres (1·9%), and this may account for the lack of an observed relationship between colonization and day-care attendance.

There is insufficient information on the epidemiology of Hib disease and carriage of the organism in children <2 years old in Turkey. This study was not prospective and did not reflect the characteristics of all children in the community. Nevertheless, to our knowledge, it is one of the few studies carried out on a large number of healthy children <2 years old. It may not be appropriate to extrapolate our findings to the general community but breastfeeding and passive smoking rates are highly representative of national practice. Since there are few data on Hib infections from developing countries, which may be due to the necessity of using selective media to recover the organism, the rate of Hib carriage found here in Turkey may indicate the true burden of this infection in the national community. Further studies are warranted in countries of similar economic status to determine whether the rates identified here are representative of the global situation.

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